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Quantitative Procedure for Gas Chromatographic Analysis of Head-Space Vapor Over Sterile Concentrated Milk [1, 2]

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Abstract

An apparatus has been designed in which it is possible to obtain the head-space vapor over sterile concentrated milk in argon under controlled conditions of volume, pressure, and temperature and from which a measured aliquot at atmospheric pressure can be withdrawn for gas-chromatographic analysis. The effect of various factors influencing the sampling and chromatographic procedures were investigated and controls established. The chromatographic assembly and conditions finally selected consisted of a 2.74-m Apiezon-L column at 50 C with a Strontium-90 detector. Five-milliliter samples were injected at an argon inlet pressure of 1.62 kg/cm^2 gauge. Water vapor in the sample had no significant effect upon the sensitivity of the detector within the range investigated. Saturation of sterile concentrated milks with sodium sulfate did not appreciably increase the concentration of volatile components in the head-space vapor.

This procedure is reproducible, with a standard deviation of ±3.86% of the measured peak height, and can demonstrate quantitative differences in composition of head-space vapors over sterile concentrated milks processed and stored under different conditions.

Since Bailey et al. (1) first reported the gas-chromatographic analysis of head-space vapor over cabbage, a number of reports (4-8, 10, 11, 13, 15, 16, 18, 19, 21-23) on the application of this techique to the odor evaluation of many food products and other odorous materials have appeared. Weurman (23) and Ozeris and Bassette (17), working with synthetic aqueous solutions of aldehydes, ketones, and esters, established a linear relationship between the heights of the peaks in the chromatogram of the head-space vapor and the concentrations of the compounds in the solutions. Bassette and co-workers (4) also demonstrated that it should be possible to correlate differences in the chromatograms of the head-space vapor over milk samples with the various flavor defects of milk. Buttery and Teranishi (7) investigated the quantitative aspects of the head-space vapor analysis and its use to follow the development of oxidative rancidity and browning in dehydrated potato.

The present work consists of the development of a precise quantitative procedure for sampling and gas-chromatographic analysis of volatiles in the vapor over sterile concentrated milk, to correlate the chromatographic profiles obtained with changes in the flavor of the product due to processing and storage.

Experimental Procedures

Manufacture of sterile concentrated milk. Milk collected from the University of Illinois dairy barns was condensed in three batches, aged for 40 hours, canned and sterilized by the conventional, high-temperature short-time and aseptic processes at the Pet Milk Company, Greenville, Illinois. The sterile milk samples were stored at 4.4, 26.7, and 37.8 C. Unless otherwise indicated, conventionally sterilized concentrated milk stored at 37.8 C for 18 months was used in this study.

Special equipment. Head-space vapor-sampling apparatus (Fig. 1).

A Jarrell-Ash Model 700 Universal Chromatograph equipped with a 20-mc Strontium-90 beta-emission detector unit and a 10-mv Leeds and Northrup Speedomax H recorder was used for the analysis. The carrier gas, argon, was passed through a tube containing a Linde 5 Å molecular sieve to remove moisture before entering the chromatographic column. The gas chromatographic column consisted of a stainless steel tube 2.74 m by 0.38 cm (id) packed with 10% Apiezon-L on Chromosorb W, 90-100

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[2] Taken from the thesis of N. R. Sundararajan, presented in June, 1964, to the University of Illinois, in partial fulfillment of the requirements for the degree of Master of Science.

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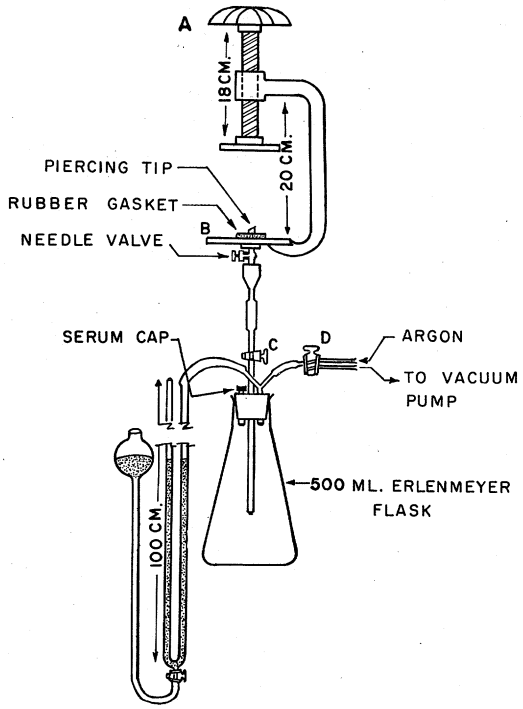

FIG. 1. Head-space vapor sampling assembly.

mesh. The fixed-ratio T type combination stream splitter and sample collector originally provided with the instrument was replaced by a 1.27-mm stainless steel tube to allow all of the injected sample to flow through the detector.

Sampling procedure. One hundred and seventy-seven grams of anhydrous Na_2SO_4, enough to supersaturate 350 ml of sterile concentrated milk, were placed in the Erlenmeyer flask and the flask stoppered with a three-holed no. 7 neoprene stopper as shown in the diagram (Fig. 1). The flask was connected to a Boerner shaker (not shown in the diagram) and immersed in a constant temperature water bath at 50 C. The needle valve was closed, Stopcock C was opened, and the flask evacuated by means of a Duo-Seal high-vacuum pump until the difference in the mercury levels in the manometer was equal to the atmospheric pressure. Stopcock D was closed and, if the difference in the mercury levels in the manometer did not change after 15 minutes, the assembly was assumed to be free of leaks. To avoid excessive foaming when the sterile concentrated milk was run into the flask, the vacuum in the flask was partially broken by argon, which had passed through a tube containing a Linde 5 Å molecular sieve until the mercury level in the closed arm of the manometer dropped about 10 cm.

A can of the sterile concentrated milk to be analyzed was placed on the rubber gasket over Platform B, and Screw A was tightened compressing the rubber gasket until the can was punctured by the piercing tip. A small hole was made on the side of the can near the top, to avoid the creation of vacuum inside the can, and the needle valve and Stopcock C were opened immediately, allowing the sterile concentrated milk to run directly into the flask. The volume of the milk was controlled to about 350 ml by filling to a predetermined mark on the flask. The needle valve and Stopcock C were then closed and argon was let into the flask until the pressure inside the flask was equal to the atmospheric pressure.

After shaking the flask for ten minutes, a 10-ml gas-tight Hamilton syringe was inserted through the serum cap and 5 ml of the head-space vapor was drawn into the syringe. Then more argon was let into the flask until the levels of mercury in the manometer were again equal. After about five minutes, the pressure in the flask was measured (p_1) and the vapor in the syringe pushed back into the flask and the syringe removed. The mercury leveling bulb was then raised until the level of mercury in the closed arm of the manometer reached the original level and the pressure in the flask determined (p_2). These steps were necessary to assure that the vapor sample removed for analysis was at atmospheric pressure and to determine the volume of the head space accurately. The head-space volume was calculated from the formula

$V = vp_1/p_2 - p_1$ where
V = volume of head space, and
$v = 5 + 0.00367\ (t_b - t_r)$ = volume of head-space vapor drawn into the syringe corrected to bath temperature.
t_b and t_r = temperature of the bath and the room, respectively.

The flask and contents were shaken for 2 hr and a 5-ml aliquot of the head-space vapor removed for gas-chromatographic analysis.

Although only Dow-Corning silicone high-vacuum grease was used in the stopcocks in this study, it was found to contain volatiles that contributed peaks to the gas chromatogram at the high sensitivity of operation. When new connecting rubber tubes and neoprene stopper were used with a minimum of grease, these contaminants were absent. However, they reappeared in time.

The teflon tip of the gas-tight syringe also

yielded a single contaminant and, therefore, before each use the syringe was flushed with argon at least three times, or until free of the contaminant. As an additional precaution, the syringe was kept periodically in a vacuum oven at 40 C.

An alternate solution to the problem of these contaminants would be to employ teflon stopcocks and connections and to allow for the presence of the contaminant from this source in the chromatograms.

Gas-chromatographic procedure. Prior to injection of the sample, the voltage of the power supply was always so adjusted that the recorder registered 10% of the full-scale deflection for the standing current at the steady state, with the range selector switch at the 10^{-7} position. The purge gas flow was kept at about 47.2 ml per minute. The removal of the combination stream splitter and sample collector and the design of the detector in the Jarrel-Ash model 700 gas chromatograph made measurement of the flow rate of the carrier gas through the column impossible. Hence, the flow rate was controlled by adjusting the argon inlet gauge pressure reading as accurately as possible. The detector, the injector, and the splitter were always maintained at 215, 200, and 300 C, respectively. The temperatures were determined accurately by means of an external potentiometer (Rubicon) and a reference junction in an ice bath at 0 C.

Results and Discussion

Figure 2 shows typical chromatograms of the head-space vapor over sterile concentrated milk. The heights of only the peaks marked 1, 2, and 3 were measured for quantitative evaluation of experimental conditions, since the other peaks were less sharp or overlapped too much with adjacent peaks to make sufficiently accurate quantitative measurements.

The initial negative detector response noticed in the chromatograms has been observed by all investigators using the argon ionization detector for head-space vapor analysis and has been attributed to the presence of air in the injected sample (19, 23). A 10-ml sample of 10:90 air-argon mixture will yield a comparable negative response. Since five successive evacuations and refillings with argon of the sample flask prior to addition of the milk did not show any sizeable decrease in the negative detector response, the dissolved gases in the milk were assumed to be responsible for the negative peak.

Effect of water vapor. While the argon-ionization detector is relatively insensitive to water, water vapor does greatly reduce its sensitivity of response to organic compounds (6, 9, 13, 19, 20). However, the nature and extent of desensitization of the detector by water vapor is not clearly known. Lovelock et al. (12) have shown that water contents of less than 30 ppm in the carrier gas do not affect the detector, but this depends upon the geometrical design of the detector and other parameters employed in the experiment. Therefore, equal amounts of 2-pentanone were injected into the sample flask containing dry argon or argon which had been slowly bubbled through water by means of a gas-dispersion tube fitted with a fritted cylinder of 40-60 μ maximum pore size. These systems were sampled in the usual manner and the 2-pentanone peak heights are reported in Table 1. No significant decrease was observed in the detector response to pentanone when water vapor was present in quantities likely to be present in the head-space vapor

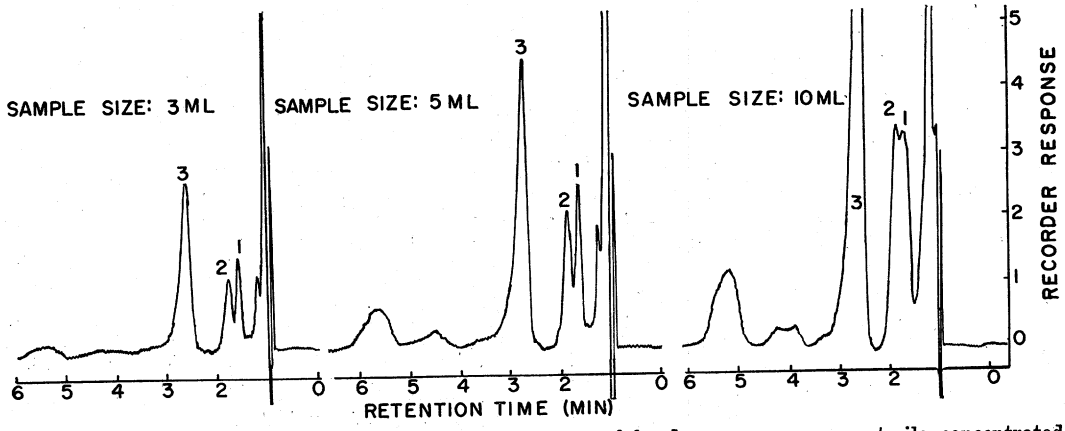

FIG. 2. Effect of sample size on the gas chromatogram of head-space vapor over sterile concentrated milk. (Oven temperature, 50 C; argon-inlet gauge pressure, 1.62 kg/cm^2; sensitivity, 10^{-9}.)

TABLE 1. Effect of water vapor on the gas-chromatographic peak height of 2-pentanone vapor. (Oven temperature, 100 C; argon-inlet gauge pressure, 2.11 kg/cm²; sensitivity, 10^{-7}; sample size 41.5 μl of 3.44% aqueous solution.)

Experiment	Dry argon	Moist argon
	(cm)	
1	3.15	2.85
2	3.15	3.15
3	3.05	3.15

Analysis of variance

Source of variance	Degrees of freedom	Mean square	Significance*
Water vapor	1	0.006666	N.S.
Error	4	0.016666	

* N.S. = not significant.

samples. Swoboda (20) has observed that when an aqueous solution of a mixture of solutes is chromatographed employing an argon-ionization detector, a solute coming off the column along with the water vapor is hardly observed, while the size of the peak for a solute coming after water is greatly diminished. Therefore, it is probable that, in the present experiment, either the water emerged from the chromatographic column after the pentanone, or the concentration of water vapor was too small to interfere with the sensitivity of the detector. If the water vapor emerged after the pentanone, which has a retention time higher than that of any of the components observed in the head-space vapor over sterile concentrated milk, it is conceivable that the detector was perhaps desensitized by the water and the components emerging from the column after water were not noticeable. If this was the case, the chromatograms of the head-space vapor obtained in the present study do not show all of the components present in the head-space vapor over sterile concentrated milk. However, no single chromatogram obtained under one set of sampling and analytical conditions can be expected to demonstrate all of the possible components present in the sample.

Effects of variables. To establish the conditions for optimum sensitivity and resolution and the control necessary for reproducible results, the effects of the following variables were investigated: saturation with sodium sulfate, gas-chromatographic sample size, column-oven temperature, argon-inlet pressure, temperature and time of equilibration, and volume of head space.

Bassette and co-workers (3, 4, 17) and Morgan and co-workers (14, 15) reported the use of salting-out procedures with anhydrous sodium sulfate to enhance the concentration of the volatiles in the head space over aqueous systems. Therefore, the effect of adding anhydrous sodium sulfate on the chromatogram of the head-space vapor over sterile concentrated milk was investigated by performing duplicate experiments in the usual manner, with and without saturation with sodium sulfate. The volume of the head space was maintained as constant as possible in both cases by varying the volume of the milk. The results in Table 2 show that the addition of sodium sulfate had very little effect, if any, on the peak heights of the components in the head space over sterile concentrated milk.

This failure of the sodium sulfate to appreciably enrich the head-space vapor could perhaps be accounted for by the large amount of fat and other milk constituents in the sterile concentrated milk which would compete with the aqueous phase for these components, since they may be dissolved in the fat or adsorbed on the other colloidal milk constituents. Some support for this explanation is found in the works of Bassette et al. (2) and Toan et al. (22), in which they observed lower peak heights for the same concentration of volatile organic substances in milk than in water or urine, even though both systems were saturated with Na_2SO_4 before head-space vapor analysis.

The size of the sample injected into the col-

TABLE 2. Effect of addition of sodium sulfate on the peak heights* of the gas-chromatographic components of the head-space vapor over sterile concentrated milk. (Oven temperature, 50 C; argon-inlet gauge pressure, 1.62 kg/cm²; sensitivity, 10^{-9}; sample size, 5 ml.)

Component	Without sodium sulfate	With sodium sulfate
	(cm)	
1	4.08	4.08
2	3.34	3.48
3	5.97	6.08

Analysis of variance

Source of variance	Degrees of freedom	Mean square	Significance**
Component (C)	2	7.369158	V.S.
Salt (S)	1	0.020009	2.5-5.0%
S × C	2	0.005158	N.S.
Error	6	0.003108	

* Average of duplicates.
** V.S. = very significant; N.S. = not significant.

umn plays a very significant role in gas chromatographic analyses. The larger the volume of the sample injected, the smaller will be the error in volume measurement and the larger will be the component peaks. On the other hand, too large a sample will overload the chromatographic column affecting resolution of the components and will exceed the linear response of the detector (12). With these principles in mind, gas chromatograms of 3-, 5-, and 10-ml samples of the head-space vapor over sterile concentrated milk were obtained in the usual manner and are shown in Figure 2. The peak heights of the components in the 3- and 5-ml samples were proportional to the sample size within the limit of experimental error. In the 10-ml sample, the peaks of Components One and Two overlapped, making quantitative interpretation difficult. These results are consistent with the observation of Mackay et al. (13) that, in the analysis of head-space vapor by gas chromatography employing an argon-ionization detector the sample size should be equivalent to no more than five seconds' flow of the carrier gas and that, under normal flow rates, the sample should be limited to 5 ml. Therefore, 5-ml aliquots were employed throughout the balance of this study. It should be pointed out, however, that within the limits indicated the sample size is dependent upon the particular material being investigated.

The results of similar studies of the effect of the other variables have been summarized in Tables 3 and 4. The temperature of the gas-chromatographic column and the flow rate of the carrier gas have a marked influence on the retention times, peak heights, and resolution of the components. The investigations of the effects of these variables indicated that a column-oven temperature of 50 C and an argon-inlet gauge pressure of 1.62 kg/cm^2 gauge gave the best resolution of the components in the head-space vapor over sterile concentrated milk.

Studies of the effects of the time and temperature of equilibration indicated that the higher the temperature, the higher the concentration of volatile components in the head space. No components were observed at 50 C that were not also present at 40 C. However, since temperatures over 50 C might produce new components, higher temperatures were not attempted.

The time required to reach equilibrium at 50 C was found to be 60 minutes. To be safe, all sterile concentrated milk samples were equilibrated for 2 hr in this study.

The concentration of the volatile components in the head-space vapor should be a function of the ratio of the volume of the head space to the total volume of the flask. If the vapor and solution involved are ideal in their behavior, and the effect of the volume of the head space upon the equilibrium pressure can be neglected, the relationship between the concentration of a volatile component in the head space to the ratio of the volume of the head space to the total volume is

$$C_g = KC_o(1 - r)/[1 + (K - 1)r]$$

where C_g = the concentration of the volatile component in the gas,

TABLE 3. Effect of variables upon peak heights of the gas-chromatographic components of the head-space vapor over sterile concentrated milk.

Variable	Range investigated	Linear regression coefficient[b]	Value selected	Control necessary[a]
Column-oven temperature	40-80 C	0.3585 cm/deg C	50 C	±0.39 C
Argon-inlet gauge pressure	1.12-2.11 kg/cm^2 Gauge	4.993 cm/kg/cm^2	1.62 kg/cm^2 Gauge	±0.030 kg/cm^2 Gauge
Temperature of equilibration	40-50 C	0.1725 cm/deg C	50 C	±0.88 C
Time of equilibration	30-120 min	.....	120 min	>60 min[c]
Volume of head space	71.03-173.0 ml	.....	150 ml	Independent[d]

[a] The control necessary so that the error introduced from this source is less than one-fourth of the mean error variance.
[b] Linear regression coefficient for Component 3, since it is most sensitive to changes in the variable. The control necessary is based on this value.
[c] Equilibrium is reached in 60 min.
[d] The peak heights are independent of the volume of the head space throughout the range investigated.

TABLE 4. Effect of variables upon the logarithm of the retention time of the gas-chromatographic components of the head space over sterile concentrated milk.

Variable	Range investigated	Regression coefficients			Value selected	Control necessary[a]
		Linear	Quadratic	Cubic		
Column-oven temperature	40-80 C	0.05351[b] deg C^{-1}	-1.290×10^{-3} [b] deg C^{-2}	8.542×10^{-6} [b] deg C^{-3}	50 C	±0.32 C
Argon-inlet gauge pressure	1.12-2.11 kg/cm^2	−0.02982 cm^2/kg	−0.06785 cm^4/kg^2	.....	1.62 kg/cm^2	±0.03 kg/cm^2

[a] The control necessary so that the error introduced from this source is less than one-fourth of the mean error variance.

[b] Regression coefficients for Component 3, since it is most sensitive to changes in the variable. The control necessary is based on these values.

K = the partition coefficient for the component,

C_o = the initial concentration of the component in the milk,

and r = the ratio of the volume of the head space to the total volume of the flask.

To establish the control necessary for this variable, the volume of the head space was varied and its effect on the peak heights of the gas-chromatographic components determined. These results suggest that within the range of ratios investigated the peak height is independent of the volume of the head space, or symbolically $K << 1$ and $C_g = KC_o$.

Application of the head-space vapor sampling procedure to comparison of sterile concentrated milks. To demonstrate that the procedures developed in this investigation are capable of indicating qualitative and quantitative differences in the composition of volatiles in different samples of sterile concentrated milk, the head-space vapors over concentrated milks sterilized by the conventional, high-temperature short-time, and aseptic processes, respectively, and stored at 4.4 and 26.7 C for 18 months were chromatographed. The chromatograms obtained are represented in Figure 3.

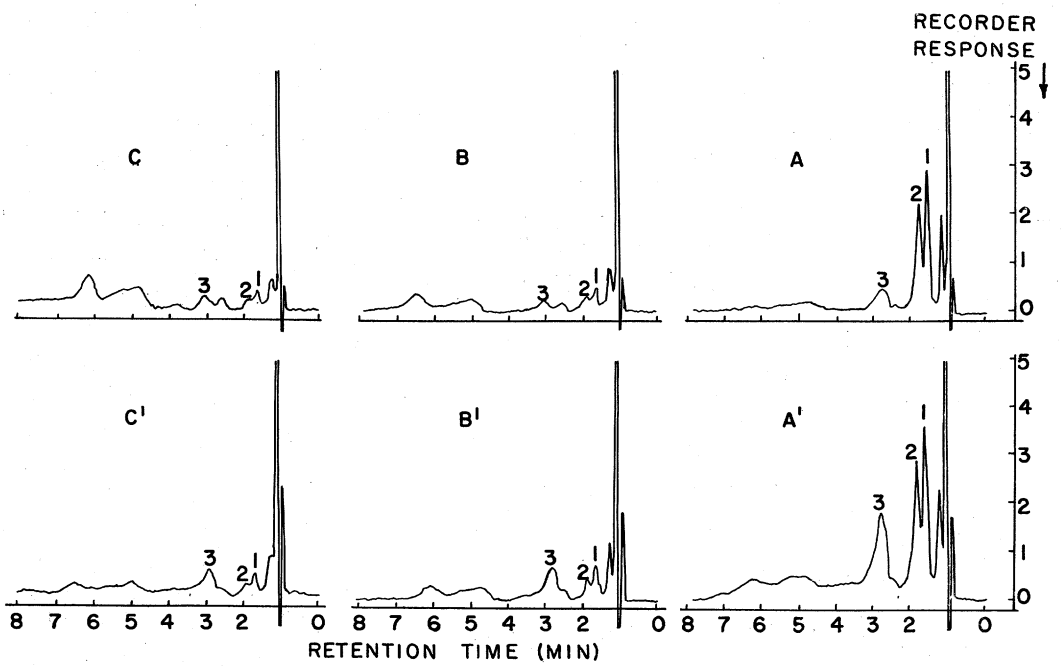

FIG. 3. Gas chromatograms of the head-space vapor over concentrated milk sterilized by the conventional (A), aseptic (B), and HTST (C) processes and stored for 18 months at 4.5 C (unprimed) and 26.7 C (primed). (Oven temperature, 50 C; argon-inlet gauge pressure 1.62 kg/cm^2; sensitivity, 10^{-9}; sample size, 5.0 ml.)

The peak heights and the logarithms of retention times of the components are given in Tables 5 and 6, respectively. Analyses of variance show that, while there is no significant difference in the retention times of the components, suggesting that the compounds investigated are the same in all the chromatograms, there are significant differences in the peak heights of the components in the head-space vapors over the different sterile concentrated milks, due both to processing procedures and to storage conditions.

With the control of the variables as specified, the procedure yields gas-chromatographic results with a mean error variance of 91.92×10^{-3} cm^2 in the peak heights, which corresponds to a standard deviation of $\pm 3.86\%$ of the peak height of Component 3 and a mean error variance of 59.52×10^{-6} in the logarithm of the retention times, which corresponds to a standard deviation of $\pm 1.78\%$. This accuracy should suffice for routine analysis of samples. When more precise results are desired, the variation in the sampling and gas chromatographic conditions can be compensated for by the incorporation of an internal standard in the head-space vapors and the peak height and retention times calculated relative to the internal standard.

TABLE 5. Comparison of the peak heights* of the gas-chromatographic components in the head-space vapors over sterile concentrated milks processed and stored under different conditions. (Oven temperature, 50 C; argon-inlet gauge pressure, 1.62 kg/cm²; sensitivity, 10^{-9}; sample size, 5 ml.)

	Conventional		HTST		Aseptic	
Component	Temperature of storage (C) 4.4	26.7	4.4	26.7	4.4	26.7
	(cm)					
1	5.11	5.88	0.71	0.76	0.85	1.22
2	4.11	4.76	0.39	0.46	0.49	0.70
3	0.92	2.92	0.56	0.88	0.29	1.14

Analysis of variance

Source of variance	Degrees of freedom	Mean square	Significance**
Component (C)	2	5.105486	V.S.
Milk (M)	2	41.798436	V.S.
Temperature (T)	1	3.180278	V.S.
C × M	4	4.320303	V.S.
M × T	2	0.844203	V.S.
C × T	2	0.483120	V.S.
C × M × T	4	0.082919	V.S.
Error	18	0.015433	

* Average of duplicates.
** V.S. = very significant; N.S. = not significant.

References

(1) Bailey, S. D., M. L. Bazinet, J. L. Driscoll, and A. J. McCarthy. 1961. The volatile sulfur components of cabbage. J. Food Sci., 26: 163.

(2) Bassette, R., S. Ozeris, E. E. Bartley, and J. S. Yadava. 1963. Analysis of biological fluids for carbon tetrachloride after its administration into the bovine rumen. J. Dairy Sci., 46: 444.

(3) Bassette, R., S. Ozeris, and C. H. Whitnah. 1962. Gas chromatographic analysis of

TABLE 6. Comparison of the logarithms* of the retention times of the gas-chromatographic components in the head-space vapors over sterile concentrated milks processed and stored under different conditions. (Oven temperature, 50 C; argon-inlet gauge pressure, 1.62 kg/cm²; sensitivity, 10^{-9}; sample size, 5 ml.)

	Conventional		HTST		Aseptic	
Component	Storage temperature (C) 4.4	26.7	4.4	26.7	4.4	26.7
1	0.2135	0.2175	0.2175	0.2174	0.2108	0.2095
2	0.2648	0.2695	0.2695	0.2672	0.2648	0.2649
3	0.4487	0.4503	0.4594	0.4548	0.4668	0.4518

Analysis of variance

Source of variance	Degrees of freedom	Mean square	Significance**
Treatment (T)	5	0.000045	N.S.
Component (C)	2	0.192675	V.S.
T × C	10	0.000042	N.S.
Error	18	0.000059	

* Average of duplicates.
** V.S. = very significant; N.S. = not significant.

head space gas of dilute aqueous solutions. Anal. Chem., 34: 1540.

(4) Bassette, R., S. Ozeris, and C. H. Whitnah. 1962. Direct gas chromatographic analysis of milk. J. Food Sci., 28: 84.

(5) Brennan, M. L., and R. A. Bernhard. 1964. Head space constituents of canned beef. Food Technol., 18: 149.

(6) Buttery, R. G., and R. Teranishi. 1961. Gas-liquid chromatography of aroma of vegetables and fruit: Direct injection of aqueous vapors. Anal. Chem., 33: 1439.

(7) Buttery, R. G., and R. Teranishi. 1963. Food vapor analysis. Measurement of fat autooxidation and browning aldehydes in food vapors by direct vapor injection gas-liquid chromatography. Agr. Food Chem., 11: 504.

(8) Day, E. A., R. C. Lindsay, and D. A. Forss. 1964. Dimethyl sulfide and the flavor of butter. J. Dairy Sci., 47: 197.

(9) Evans, R. S. 1961. Design and application of high sensitivity gas chromatographs. Journees Intern. Etude Methodes Separation Immediate Chromatog., Paris. pp. 241-247.

(10) Kepner, R. E., H. Maarse, and J. Strating. 1964. Gas chromatographic head space techniques for the quantitative determination of volatile components in multicomponent aqueous solutions. Anal. Chem., 36: 77.

(11) Loney, B. E., R. Bassette, and G. M. Ward. 1963. Some volatile components in milk, blood, and urine from cows fed silage, bromegrass and hay and grain. J. Dairy Sci., 46: 922.

(12) Lovelock, J. E., A. T. James, and E. A. Piper. 1959. A new type of ionization detector for gas chromatography. Ann. N. Y. Acad. Sci., 72: 720.

(13) Mackay, D. A. M., D. A. Lang, and M. Berdick. 1961. Objective measurements of odor. Ionization detection of food volatiles. Anal. Chem., 33: 1369.

(14) Morgan, M. E., and E. A. Day. 1965. Simple on-column trapping procedure for gas chromatographic analysis of flavor volatiles. J. Dairy Sci., 48: 1382.

(15) Morgan, M. E., and R. L. Pereira. 1963. Identity of grassy aroma constituents of some green forages. J. Dairy Sci., 46: 613.

(16) Nawar, W. W., and I. S. Fagerson. 1962. Direct gas chromatographic analysis as an objective method of flavor measurement. Food Technol., 16: 107.

(17) Ozeris, S., and R. Bassette. 1963. Quantitative study of gas chromatographic analysis of head space gas of dilute aqueous solutions. Anal. Chem., 35: 1091.

(18) Reddy, M. C., R. Bassette, G. Ward, and J. R. Dunham. 1967. Relationship of methyl sulfide and flavor score of milk. J. Dairy Sci., 50: 147.

(19) Self, R., D. G. Land, and J. C. Case. 1963. Gas chromatography using capillary column units for flavor investigation. J. Sci. Food Agr., 14: 209.

(20) Swoboda, P. A. T. 1960. The analysis of dilute aqueous solutions by gas chromatography. Chem. and Ind., 1960: 1262.

(21) Teranishi, R., R. G. Buttery, and R. E. Lundin. 1962. Gas chromatography. Direct vapor analysis of food products with programmed temperature control of dual columns with dual flame ionization detectors. Anal. Chem., 34: 1033.

(22) Toan, T. T., R. Bassette, and T. J. Claydon. 1965. Methyl sulfide production by *Aerobacter aerogenes* in milk. J. Dairy Sci., 48: 1174.

(23) Weurman, C. 1961. Gas-liquid chromatographic studies on the enzymatic formation of volatile compounds in raspberries. Food Technol., 15: 531.